REMARKS

Claim Amendments

Claim 1 has been amended to incorporate the language of claims 3, 6 and 7.

In view of the amendment to claim 1, claims 3, 6 and 7 have been canceled, and claims 4 and 8-10 have been amended accordingly.

Claim 19 has been amended to incorporate the language of claims 26 and 28, and to specify the lack of plant hormones and presence of glucose in the media of steps (b) and (d)-(g) similar to amended claim 1.

In view of the amendment to claim 19, claims 26 and 28 have been canceled, and claims 27, 29 and 30 have been amended accordingly.

Claims 20 and 23 have been amended to incorporate the language of claims 21 and 24, respectively. These claims have further been amended to specify that the medium does not contain plant hormones. Support for this latter amendment can be found at page 11, lines 5-8.

In view of the amendment to claims 20 and 23, claims 21 and 24 have been canceled, and claims 22 and 25 amended accordingly.

New claims 31-36 have been added. Support for these claims can be found at page 9, lines 28-32.

It is submitted that these amendments do not constitute new matter, and their entry is requested.

The Invention

The present invention is directed to a method of producing transgenic cotton plants which has been developed for the generation of transgenic cotton plants of many different cotton varieties and to increase the transformation efficiency and survival rate. As disclosed in the present application, the steps and the media have been developed and selected to maximize the efficiency of regeneration of *Agrobacterium* transformed cotton cells. *See*, pages 9-11 of the instant application. The method involves the use of specified plant hormones only in the callus induction

medium with no plant hormones being used in any of the other media. The method also involves the use glucose as the sole carbon source in all of the media except the plant growth medium which utilizes both glucose and sucrose. Finally, the method involves the use of asparagine and/or glutamine as a nitrogen source in the embryoid germination medium. The method of the present invention has been developed to look after the requirements for embryogenesis at the various stages of the process.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1-2, 6-11, 13-14, 18-20, 23 and 26-30 under 35 U.S.C. § 103(a) as being obvious over Rangan (5,244,802) in view of Gawel et al. (*Plant Cell Tissue Organ Develop* **23**:201-204, 1990) and further in view of Price et al. (*Plant* **145**:305-307, 1979). The Examiner cites Rangan for disclosing a process in which (i) callus is induced from cotyledon or hypocotyl explants on a medium containing glucose, kinetin and NAA, (ii) embryogenic callus is formed by growing the callus on medium containing a cytokinin and NAA, (iii) embryogenic callus is developed in suspension culture using a medium containing sucrose and NAA over 5 to 36 days, (iv) embryoids are formed on a solid medium and (v) germinating the embryoids on a medium containing 500 mg/l casein hydrolysate and 1.2 g/l KNO_3. The Examiner also cites Rangan for disclosing transformation of the explants by exposing the explants to *Agrobacterium* in a medium containing NAA. Finally, the Examiner notes that Rangan does not teach the use of petioles as the explant, the use of 2,4-D for callus initiation, the use of suspension culture in embryogenic callus formation, the lack of hormones in the exposing, selection, embryogenic callus formation and embryoid formation media and the use of 3.8 KNO_3 and/or 500 mg/l asparagine and/or 1 g/l glutamine in the germinating medium.

The Examiner cites Price et al. for disclosing the culturing of callus in a hormone-free suspension culture to induce formation of embryogenic callus and embryoids. The Examiner cites Gawel et al. for disclosing the use of cotton petiole as the explant, the use of 2,4-D and kinetin for

callus initiation and the culturing of callus in suspension culture to induce formation of embryogenic callus and embryoids in which suspension culture was preferable to a solid medium.

In view of these references, the Examiner contends that it would have been obvious to modify the transformation method of Rangan to (i) use petioles as the explant, (ii) use a suspension culture in the embryogenic callus formation step, (iii) to use media lacking hormones in the exposing, selection, embryogenic callus formation and embryoid formation steps and (iv) to use 3.8 g/l KNO_3 and/or 500 mg/l asparagine and/or 1 g/l glutamine in the germinating medium. Applicants submit that the Examiner is in error in this rejection, particularly with respect to the amended claims.

Initially, Applicants note that Rangan does not disclose the use of 1.2 g/l KNO_3 in the germinating medium. Instead, Rangan discloses the use of 1.2 g/l NH_4NO_3. *See*, the Beasley and Ting medium composition in the table at the bottom of column 6. This medium also contains 25.275 g/l KNO_3, significantly more than that used in the present invention. In addition, Applicants were not able to find any disclosure in Rangan of performing a suspension culture for 5 to 36 days as stated by the Examiner. Instead, the growth is for about 3 to 4 weeks. After this time, the suspension culture is filtered to remove large cell clumps and then further cultured for 3 to 4 weeks. The large cell clumps were cultured on callus growth medium to produce somatic embryos. *See*, Example 6. The steps of filtering and further culturing can be repeated. Furthermore, the suspension culture is for proliferating the embryogenic callus and not for inducing formation of embryogenic calli as set forth in the claimed subject matter. *See*, column 9, lines 46-51.

According to Rangan, callus is induced from hypocotyls and/or coyledons on a first callus medium that contains NAA and kinetin as plant hormones and glucose as a carbon source. The callus is then subcultured on the first callus growth medium. Subsequently, the callus is cultured on a second callus growth medium. The second callus growth medium contains NAA and optionally cytokinin as plant hormones. Glucose is used as the carbon source until the secretion of phenolics has ceased after which sucrose is used as the carbon source in this second callus growth medium. The callus is subcultured on the second callus growth medium to produce embryogenic callus and somatic embryos. *See*, column 8, line 20 - column 9, line 13. Thus, Applicants submit that Rangan

teaches using sucrose as the carbon source for the initiation of embryogenic callus. According to Rangan, somatic embryos are cultured on an embryo germination medium which is rich in nitrogen usually in the form of ammonia or its equivalents. *See*, column 9, lines 19-16. As an alternative, the embryogenic callus is proliferated in suspension culture in a medium that contains NAA as a plant hormone. The medium may be the second callus medium. *See*, column 9, lines 46-61. The second callus growth medium used for the suspension culture should contain sucrose as the carbon source since the secretion of phenolics should have ceased prior to the initiation of the suspension culture. Typically after growth in suspension culture, the cells suspension is plated on either a medium that contains NAA as a plant hormone or a medium containing casein hydrolysate and a source of nitrogen to grow the embryogenic callus and develop somatic embryos.

Rangan also discloses that the method can be used in producing transgenic cotton plants by first culturing the cotyledon and/or hypocotyl segments with *Agrobacterium*. These *Agrobacterium* cultured explants are then cultured on a medium containing NAA as the plant hormone to generate callus. The transformed callus is selected by culturing on a medium containing NAA as the plant hormone. The selected transformed callus is then cultured as described above to produce embryogenic callus, embryos and plants. See, column 10, line 36 - column 11, line 2. As shown in this discussion, Rangan describes a specific process to produce transgenic cotton plants which is entirely different than the claimed subject matter of the present application. Applicants submit that there is no teaching or suggestion in Rangan to use the specific steps as set forth in the present claims with the specified media components to produce transgenic cotton plants.

Gawel et al. only relates to somatic embryogenesis and does not describe a method that includes transformation. It was well known in the art that *Agrobacterium* transformation compromised the plant tissue being transformed and simple use of methods for somatic embryogenesis was generally insufficient to regenerate transformed plant tissue. Gawel et al. discloses inducing callus tissue on a medium that contains either (1) NAA and kinetin as the plant hormones or (2) 2,4-D and kinetin as the plant hormones. Callus produced on either of these media were then cultured either on a semi-solid medium or in suspension culture using an "embryo

proliferation medium" which is hormone-free. The culturing was for 8 weeks. According to Gawel et al., the initiation medium (i.e., callus induction medium) supported embryogenesis. *See*, page 202, left column top. A copy of the paper referenced in this portion of the text (Gawel et al., *Plant Cell Reports* **5**: 457-459, 1986) is submitted herewith for the convenience of the Examiner. This paper clearly shows that callus and embryogenic callus is induced on the same medium, and thus plant hormones were used for both callus induction and embryogenic callus induction. Thus, Gawel et al. teaches that callus and embryogenic callus are both induced on the same medium using plant hormones. Consequently, the callus cultured in suspension culture was embryogenic callus and the suspension culture was not used to induce formation of embryogenic callus.

Price et al. only relates to somatic embryogenesis and does not describe a method that includes transformation. It was well known in the art that *Agrobacterium* transformation compromised the plant tissue being transformed and simple use of methods for somatic embryogenesis was generally insufficient to regenerate transformed plant tissue. Price et al. teaches that subculturing the callus on a medium containing 2iP and NAA is required for the induction of embryoids in suspension culture. In addition, Price et al. teaches that glutamine is required in the suspension culture and subsequent medium in order to produce somatic embryos. *See*, the abstract and page 306. According to the abstract this culturing is for 3-4 weeks, although the methods indicate culturing for 6-14 days and then filtering through a cheese cloth. Price et al. specifically teaches that the embryoids failed to develop into plants when transferred to the medium described by Steward and Hsu (*Planta* **137**:113-117, 1977) for the germination of excised cotton embryos. *See*, paragraph bridging pages 306 and 307. The most likely reason could be due to the poor quality of embryoids. This is the exact reason that prompted Applicants to optimize an embryoid maturation and regeneration medium (MMS3).

In the instant patent application, Applicants have described two key features of the invention. The first one is that after transformation and generation of callus, the calli were transferred to the liquid MMS2 medium and incubated for less than 20 days, preferably for 10-14 days, and then the friable cream-colored granular calli were selected and transferred for culture in solid MMS2

medium. Applicants found that a short time suspension culture treatment was very important not only for improving the frequency of embryogenesis but also the quality of embryoids. The second feature is to use asparagine and/or glutamine, preferably both asparagine and glutamine, as a nitrogen source in MMS3 medium for supporting embryoid maturation and regeneration. In this medium, Applicants found that the growth of non-embrygenic callus was inhibited, whereas the maturation and germination of embryoids, and root development of the plantlets were promoted. These key features are not described or suggested in the cited prior art.

In summary, Rangan discloses two transformation techniques. In the first technique, callus in initiated from cotyledon or hypocotyl tissue on medium containing kinetin, NAA and glucose. This callus is then cultured on medium containing a cytokinin and NAA to induce embryogenic callus. A suspension culture of the embryogenic callus is then prepared using a medium containing NAA and sucrose. The suspension culture is then transformed using *Agrobacterium* and processed further to produce transgenic plants. In the second technique, the cotyledon or hypocotyl explants are transformed with *Agrobacterium*. The explant material is then cultured on medium containing NAA to induce callus formation and then selected on the same medium containing a selection agent. Transformed callus tissue is then cultured and the embryos developed. Part of this development may be to form a suspension culture of the transformed embryogenic callus.

Because of the well-known issue of plant cell viability following *Agrobacterium* infection, Applicants do not believe that a skilled artisan would reasonably expect that the simple application of somatic embryogenesis techniques could be readily combined with transformation protocols to derive a transformation such as the Examiner has done in the formulation of the present rejection. The very art cited by the Examiner shows the unpredictability in the art. For example, Price et al. teaches that subculturing on a medium containing 2iP and NAA is absolutely required in order to obtain embryoids in the subsequent suspension culture. Price et al. also shows that glutamine is required in the medium used to produce somatic embryoids from the suspension cultured material. Thus, the combination of Price et al. with Rangan would require the use of 2iP and NAA in a method which includes suspension culture in order to achieve embryoid formation. The presently

claimed subject matter does not use such hormones as taught by Price et al. Thus, Applicants submit that the amended claims are not obvious from the prior art cited by the Examiner.

In view of the above amendments and remarks, Applicants submit that Rangan, Gawel et al. and Price et al. do not render obvious the claimed subject matter. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 3-5, 21-22 and 24-25 under 35 U.S.C. § 103(a) as being obvious over Rangan in view of Gawel et al., further in view of Price et al. and further in view of Tull et al. (US 6,242,257). The Examiner cites Tull et al. for the use of glucose as the sole carbon source in all media and the use of both glucose and sucrose as the carbon source in the "regenerating media." The Examiner contends that it would have been obvious to modify the modified Rangan process (as discussed above) to use glucose as the sole carbon source in all media or to use both glucose and sucrose in the "regenerating media." Applicants submit that the Examiner is in error in this rejection, particularly with respect to the amended claims.

Tull et al. relates to a process for the organogenesis of cotton. Applicants submit that it is well known in the art that organogenesis and embryogenesis are two entirely different techniques for generating plants in tissue culture, as confirmed by Tull et al. which states,

> Organogenesis leads to organ formation i.e., shoot (or root), which can be isolated to induce development of roots (or shoots) to produce full plant while somatic embryogenesis leads to the development of somatic embryos (embryos developed without fertilization) which have both shoot and root initials and are capable of developing into whole plant.

See, column 1, lines 60-66. The skilled artisan knows that techniques that are useful for one technique are not necessarily useful for the other technique. In fact, Tull et al. states,

> Although the ability of individual parts of plants and cells to regenerate into complete plants (called totipotency) is a well-known phenomenon, each plant or plant part requires specialized studies to invent the conditions that allow such regeneration. Some of the broadly applicable factors controlling growth

> and differentiation of such cultures have been determined. The establishment of interactions among different groups of phytohormones and growth regulators alone or in combinations are responsible for certain interrelations existing among cells, tissues and organs. There seems to be consensus that the success in inducing differentiation depends upon the type of explant, physiological condition of the explant and physical and chemical milieu of the explant during culture. Due to this, the science of tissue culture has been directed to optimize the physiological conditions of source plant, the type of explant, the culture conditions and the phytohormones used to initiate tissue culture. **This substantiates the fact that development of a new process for proliferation of plants by tissue culture is not obvious.**

See, column 1, line 66 - column 2, line 18 (emphasis added).

In view of the well-known differences between organogenesis and embryogenesis, Applicants submit that a skilled artisan would not combine Tull et al. with the other cited references as the Examiner has done, especially in view of the explicit disclosure in Tull et al. for the desire to develop an efficient process for the generation of a large number of shoots from a new tissue explant. Although the Examiner asserts that Tull et al. discloses using both glucose and sucrose in the "regenerating media," it is clear that Tull et al. only discloses the use of **either** sucrose **or** glucose and prefers glucose, even in the rooting medium. *See*, column 12, lines 63-65, column 18, lines 36-53 and claim 6. In addition, both steps of inducing shoot formation and root formation are carried out in the presence of plant hormones. *See*, column 11, line 60 - column 12, line 20. Thus, Tull et al. discloses the use of plant hormones and glucose or sucrose, but not both, as the carbon source for both shoot development and root development. According to the amended claims, embryoid germination and the growth of the young plants are performed on media that do not contain plant hormones. The media for young plant growth contains both glucose and sucrose which is not taught or suggested in Tull et al. Thus, Applicants submit that the claimed subject matter is not obvious from the teachings of Rangan, Gawel et al., Price et al. and Tull et al.

In view of the above amendments and remarks, Applicants submit that Rangan, Gawel et al., Price et al. and Tull et al. does not render obvious the claimed subject matter. Withdrawal of this rejection is requested.

Conclusion

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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